【 CASE REPORT 】

Renal Damage in Recurrent Atypical Hemolytic Uremic Syndrome Associated with *C3* p.Ile1157Thr Gene Mutation

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Abstract:

Patients with atypical hemolytic uremic syndrome (aHUS) associated with a *C3* p.Ile1157Thr mutation show a relatively high renal survival and low mortality rates, but renal histopathological findings after recurrence have been rarely reported. A 30-year-old man with a *C3* p.Ile1157Thr mutation experienced a third recurrence of thrombotic microangiopathies with neurological and gastrointestinal disorders. A renal biopsy performed during the recovery phase of acute kidney injury revealed collapsed glomeruli and arteriolar vacuolization. Approximately 10% of glomeruli were globally sclerotic, despite the absence of arterio-/arteriolosclerosis. These findings suggest substantial progression of irreversible injuries in multiple organs, including kidneys, which occurs in aHUS patients with repeated thrombotic microangiopathies.

Key words: atypical hemolytic uremic syndrome, C3 gene mutation, renal histopathology, thrombotic microangiopathy

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Introduction

Atypical hemolytic uremic syndrome (aHUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI) and is induced by the overactivation of the alternative pathway of the complement system (1). A variety of mutations in genes coding for complement factor H (CFH), complement factor I, complement factor B, complement C3, membrane cofactor protein (MCP), and thrombomodulin have been identified as pathogenic factors that trigger the activation of the complement cascade.

C3 gene mutations are primarily located in the binding regions of C3b that interact with CFH, MCP, and complement receptor 1, which inactivate C3b (2). These mutations result in dysregulation of the alternative complement pathway. Generally, the prognosis of patients with C3 gene mutations is poor; patients typically develop severe symptoms impacting multiple organs, including the kidney. One-half to two-thirds of patients progress to end-stage renal disease within the first year of the initial clinical presentation.

Interestingly, C3 gene mutations in aHUS patients have been more frequently identified in Japan (31%) than in Western countries (2-8%) (3). However, Japanese patients with C3 gene mutations show a better prognosis than those in Western countries, with the p.Ile1157Thr mutation being the most frequently identified (3, 4). This mutation is located at the interface between C3b and the short consensus repeat (SCR) 1-4 of CFH and restricts the binding of C3b to SCR1-4 of CFH (5). Although aHUS associated with the *C3* p.Ile1157Thr mutation is likely to relapse, the renal survival is relatively high, and mortality rates are low (3, 4). However, the histopathological findings of the kidney in recurrent aHUS have rarely been reported.

We herein report a case of aHUS with a *C3* p.Ile1157Thr mutation that underwent a renal biopsy during the recovery phase of AKI induced by aHUS.

Case Report

A 30-year-old Japanese man presented with a fever and sore throat, followed by abdominal pain and diarrhea. He had previously experienced thrombotic microangiopathy

Table. Laboratory Data on Admission.

Blood				Value	Normal range
	Value	Normal range			
WBC count, /μL	12,600	3,300–8,600	IgG, mg/dL	887	861–1,747
RBC count, /×10⁶ μL	3.62	4.35–5.55	IgA, mg/dL	147	93–393
Schistocyte count, %	2.9		IgM, mg/dL	101	33–183
Hemoglobin, g/dL	10.6	13.7–16.8	Haptoglobin, mg/dL	22	66–218
Platelet count, ×10³ /μL	24	158–348	Antinuclear antibody	negative	
AST, U/L	43	13–30	MPO-ANCA, U/mL	<1.0	
ALT, U/L	14	10–42	PR3-ANCA, U/mL	<1.0	
LDH, U/L	1,312	124–222	Anti-DNA antibody	negative	
Total bilirubin, mg/dL	2.8	0.4–1.5	Anti-Scl70 antibody	negative	
Alkaline phosphatase, IU/L	195	106–322	Anti-centromere antibody	negative	
Total protein, g/dL	6.3	6.6–8.1	Anti-cardiolipin-β2-glycoprotein I complex antibody, U/mL	negative	
Albumin, g/dL	3.9	4.1–4.5	Anti-Epstein-Barr virus nuclear antigen antibody	10 (+)	<10
BUN, mg/dL	36	8–20	Anti-cytomegalovirus IgM	negative	
Creatinine, mg/dL	4.01	0.65–1.07	Anti-varicella-zoster virus IgM	negative	
Uric acid, mg/dL	8.7	3.7–7.0	Anti-varicella-zoster virus IgG	54.7 (+)	<2
Sodium, mEq/L	132	138–145	Anti-human immunodeficiency virus antigen/antibody	negative	
Potassium, mEq/L	3.5	3.6–4.8	Hepatitis B virus surface antigen	negative	
Chloride, mEq/L	99	101–108	Anti-hepatitis C virus antibody	negative	
Calcium, mg/dL	8.1	8.8–10.1	PT-INR	1	
CRP, mg/dL	18.32	<0.14	aPTT, sec	33.4	24–36
C3, mg/dL	89	73–138	Fibrinogen, mg/dL	459	150–400
C4, mg/dL	26	11–31	Direct Coombs	negative	
CH50, U/mL	49.4	30–50	Indirect Coombs	negative	

Urine			
	Value		Value
pH	6	Occult blood	3+
Specific gravity	1.009	Urinary protein creatinine ratio, g/gCr	1.94
Protein	2+	RBC, /high power field	5–9
Glucose	negative	WBC, /high power field	1–4

AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, BUN: blood urea nitrogen, C: complement, CRP: C-reactive protein, Ig: immunoglobin, MPO/PR3-ANCA: myeloperoxidase/proteinase 3-anti-neutrophil cytoplasmic antibody, PT-INR: prothrombin time-international normalized ratio, aPTT: activated partial thromboplastin time: RBC: red blood cell, WBC: white blood cell

(TMA) on three occasions. The patient had no family history of TMA.

At eight years old, he had suffered the first TMA-associated symptoms, including AKI. He had been diagnosed with HUS because verotoxin was detected and received treatment, including transfusion of plasma and platelets together with antibiotics. The peak serum creatinine concentration (sCr) in the first episode was 1.6 mg/dL. At 26 and 28 years old, he presented with clinical features of TMA and AKI again, with peak sCr values of 1.6 mg/dL and 2.7 mg/dL, respectively. In the third episode, he underwent plasma infusion. In each episode, his renal function subsequently returned to the normal range (sCr 0.9 mg/dL).

On an examination, his vital signs were as follows: body temperature (39.7℃), blood pressure (102/52 mmHg), and heart rate (96 bpm). The patient was conscious and lucid. His tonsils were inflamed, red, and swollen. There were no particular findings of interest in other parts of the body. Laboratory findings confirmed hemolytic anemia, thrombocytopenia, and a reduced renal function with microscopic hematuria and proteinuria (Table). Hypocomplementemia was not identified. Computed tomography of the neck, chest, and abdomen detected no apparent evidence of tumors or infections other than tonsillitis. *Streptococcus pyogenes* was identified from his tonsils, but cultures from his stool, blood, and urine were negative. Acute tonsillitis was initially treated with ceftriaxone and then changed to ampicillin after the pathogen was detected (Fig. 1).

Although therapies with antibiotics and plasma transfusion improved the hemolytic anemia, thrombocytopenia, as well as pyrexia and inflammation of the tonsils, the patient showed central nervous system (CNS) symptoms, including headache, seizure, and visual disorder from day 5 and mild hypertension (blood pressure of 150-165/70-90 mmHg) from
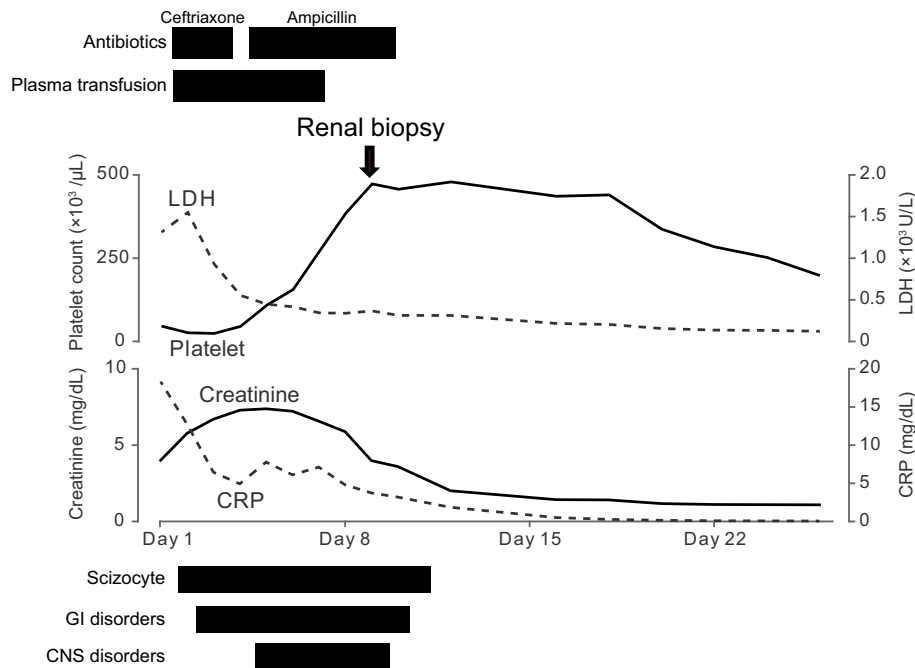
Figure 1. Clinical course. CNS: central nervous system, CRP: C-reactive protein, GI: gastrointestinal, LDH: lactate dehydrogenase

day 8 of hospitalization.

Magnetic resonance imaging of the brain on day 8 revealed posterior reversible encephalopathy syndrome. Although his sCr rapidly increased to 7.4 mg/dL on day 5, the values tended to decrease subsequently. Microscopic hematuria, abdominal and CNS disorders finally disappeared by day 10. Hypertension was treated with 20 mg/day of oral sustained-release nifedipine from day 8 to 12, and then his blood pressure returned to normal without antihypertensive agents.

A percutaneous renal biopsy was performed on day 9, during the recovery phase of AKI when his sCr was still elevated (4.0 mg/dL). The specimen contained 52 glomeruli, 6 of which were globally sclerotic. Most of the glomeruli were collapsed (Fig. 2A). Neither crescent formation nor fibrin thrombosis was identified in the glomeruli. There was focal tubular atrophy and interstitial edema with the infiltration of lymphocytes. Although no sclerotic changes or fibrin thrombi were observed in the renal microvessels, vacuole formation was observed in the wall of the interlobular arteries and arterioles, and some sclerosed glomeruli were associated with arterioles showing vacuolization. (Fig. 2B, C). An immunofluorescence analysis revealed negative staining with immunoglobulin G (IgG), IgM, IgA, C3, and C1q in the glomeruli. Electron microscopy demonstrated mild endothelial injury that was evidenced by enlarged endothelial cells, subendothelial lucency, and focal podocyte effacement. Electron-dense deposits were not identified (Fig. 2D).

Given his normal serum ADAMTS13 activity and negative studies associated with TMA, including stool culture, O-157 lipopolysaccharide (LPS) antigen or antibody, and other possible causes, such as drugs, malignancies, collagen diseases, and viral infection (Table), he was clinically suspected of having aHUS. The administration of antibiotics and plasma transfusion for six days effectively improved his clinical symptoms; therefore, additional therapies, such as plasma exchange or the administration of eculizumab, were not applied.

Later, a heterozygous *C3* p.Ile1157Thr mutation was detected, which led to the definitive diagnosis of aHUS associated with a C3 gene mutation. Increased plasma Ba, C5a, and sC5b-9 [2,838.6 ng/mL (normal range: 275.6-685.2), 24.12 ng/mL (0.20-15.62), and 581.4 ng/mL (37.0-260.6), respectively] on day 4 were consistent with the diagnosis. A genetic analysis also detected heterozygous *CFH* p.Tyr1058His and p.Val1060Leu, which were previously reported in aHUS patients (6). However, the pathogenicity of these variants is unknown, and they were classified as likely benign variants in a recent study (7). The renal function and levels of Ba, C5a, and sC5b-9 returned to the normal ranges at 4 and 8 months, respectively. The latest renal data at 2 years' follow-up showed an sCr of 0.76 mg/dL, estimated glomerular filtration rate of 96 mL/min/1.73 m^2, and urinary protein creatinine ratio of 0.04 g/gCr.

Discussion

We encountered a case of recurrent aHUS associated with a heterozygous *C3* p.Ile1157Thr mutation, in which the renal histopathology was evaluated during the recovery phase of AKI. A renal biopsy revealed collapsed glomeruli, glomerular endothelial damage, and vacuolization of microarteries. Although a normal renal function was regained following treatment in every recurrent episode, the renal bi-
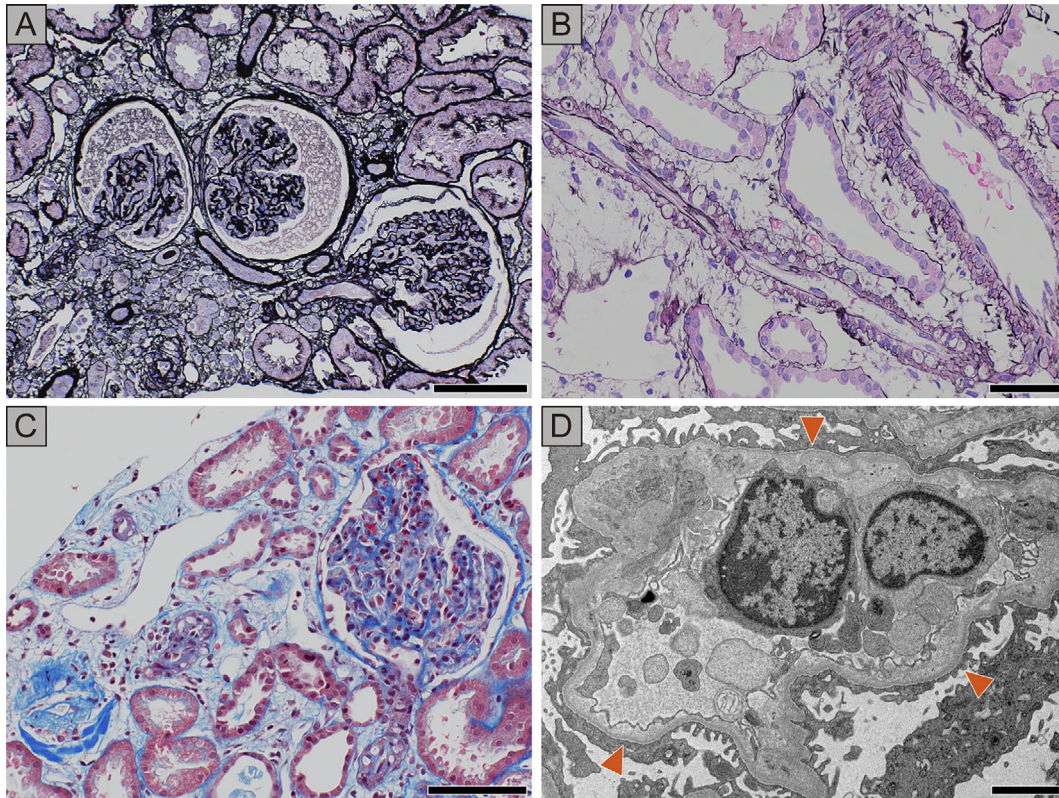
Figure 2. Findings of renal biopsy. A: The glomeruli are collapsed, and wrinkling of the capillary tuft can be seen. Focal tubular atrophy and interstitial edema are noted. Periodic acid silver methenamine-Hematoxylin and Eosin (PASM-H&E) staining, scale bar=100 μm. B: Myocyte intracytoplasmic vacuoles are visualized in the intralobular arteries and arterioles. PASM-H&E staining, scale bar=50 μm. C: Masson's trichrome staining demonstrates vacuolization of the arterioles, while thrombosis is not apparent. Scale bar=100 μm. D: The ultrastructural analysis shows swelling of endothelial cells and widening of the subendothelial area (arrowheads) filled by electron-lucent material with wrinkling of the glomerular basement membrane and mesangial interposition. Focal podocyte foot process effacement is seen around these lesions. Electron-dense deposits are not observed. Scale bar=2.5 μm.

opsy findings revealed that approximately 10% of the glomeruli were globally sclerotic, despite the absence of arterio- and arteriolo-sclerosis. These renal histopathological findings suggest substantial renal damage resulting from repeated vascular injuries associated with aHUS.

The present case was clinically diagnosed with aHUS based on microangiopathic hemolytic anemia, thrombocytopenia, and AKI in the absence of decreased ADAMTS13 levels, verotoxin, or any other possible causes of secondary TMA. The diagnosis was subsequently confirmed by a genetic analysis, which revealed a heterozygous *C3* p.Ile1157Thr mutation, a well-known pathogenic mutation that causes overactivation of the complement pathway (3). The Ba, C5a, and sC5b-9 levels, which had been significantly elevated, were ameliorated after the treatment, further indicating that acute activation of the alternative complement pathway played a pathogenic role in this case (8).

Renal biopsy findings revealed glomerular endothelial damage and collapsed glomeruli. We could find no reason other than aHUS for the endothelial damage. Notably, vacuole formation was observed in the wall of the interlobular arteries and arterioles. Arteriolar vacuolization sometimes occurs in acute angiopathy of calcineurin inhibitors (CNIs); however, it is not specific to CNI toxicity (9). This lesion reflects direct toxic effects on the arterial wall or vasoconstriction or both (10), and extensive endothelial injury occasionally causes TMA (11). The complement activation product C5a can induce vasoconstriction in intrarenal arteries and arterioles (12-14). Therefore, the arteriolar vacuolization observed in our case might be a result of complement-mediated arterial damage. Although no remarkable thrombi were identified in the glomeruli or arteries in the renal tissue, thrombi are not always present in aHUS cases (15). One possible reason that no thrombosis was observed in the renal tissue specimens could be due to a sampling error; collapsed glomeruli implied stenosis or obstruction of the intrarenal arteries, which might not have been included in the specimen. Another possible reason is the timing of the renal biopsy, as the renal biopsy was performed four days after the sCr levels peaked in the recovery phase of AKI; therefore, modest thrombosis might have already disappeared. In the present case, the findings in the renal mi-

crovasculature were mild, which may be reflective of the better renal outcomes observed in cases of aHUS with a *C3* p.Ile1157Thr mutation than in those with other causes, which frequently display severe TMA lesions.

The present case showed CNS involvement, including a headache, seizure, and visual disorders, which are extremely common in aHUS patients (16). In a Turkish registry of pediatric aHUS patients, seizure and visual loss were observed in 20.7% and 2.3% of patients, respectively (17). Magnetic resonance imaging of the brain revealed posterior reversible encephalopathy syndrome, which is sometimes observed in aHUS patients (17). The patient also presented with gastro-intestinal disorders including abdominal pain and diarrhea, which are commonly involved in aHUS (16). These findings suggest that complement-mediated microvascular injuries might have occurred in multiple organs in addition to the kidney in the present patient.

In our case, treatment with plasma transfusion and antibiotics effectively improved the symptoms associated with TMA. Most cases of aHUS with a *C3* p.Ile1157Thr mutation respond to plasma therapy, such as plasma transfusion and plasma exchange, or preserved therapy at the initial onset (3). Cases with frequent recurrence may be treated with eculizumab, a humanized monoclonal antibody to C5. Thus, eculizumab may be an effective treatment for highly recurrent aHUS associated with a *C3* p.Ile1157Thr mutation. In fact, it has been reported that an aHUS patient with a *C3* p.Ile1157Thr mutation achieved successful remission with eculizumab but suffered recurrence four months after eculizumab was discontinued (18). Eculizumab may be a preferable treatment for our case in order to reduce the risk of recurrent episodes of complement activation and further progression of the multiple organ injuries.

In summary, we reported a case of recurrent aHUS with a heterozygous *C3* p.Ile1157Thr mutation. The coexistence of acute and chronic histopathological lesions of the kidney suggests substantial progression of irreversible injuries of multiple organs, including the kidneys, in patients with aHUS showing repeated clinical presentations of TMA. The present case suggested that multiple organ damage in aHUS can occur even if patients respond positively and appear to have recovered in response to the treatment.

The authors state that they have no Conflict of Interest (COI).

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